

Sulfate Reducers Can Outcompete Methanogens at Freshwater Sulfate Concentrations†

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Acetate and hydrogen metabolism by sulfate reducers and methanogens in the profundal sediments of an oligotrophic lake were examined. Inhibition of sulfate reduction with molybdate stimulated methane production from both hydrogen and acetate. Molybdate did not stimulate methane production in sediments that were preincubated to deplete the sulfate pool. Sulfate reduction accounted for 30 to 81% of the total of terminal metabolism proceeding through sulfate reduction and methane production in Eckman grab samples of surface sediments. The ability of sulfate reducers to effectively compete with methanogens for acetate was related to the sulfate reducers' lower half-saturation constant for acetate metabolism at in situ sulfate concentrations. Processes other than sulfate reduction and methanogenesis consumed hydrogen at elevated hydrogen partial pressures and prevented a kinetic analysis of hydrogen uptake by sulfate reducers and methanogens. The demonstration that sulfate reducers can successfully compete with methanogens for hydrogen and acetate in sediments at in situ sulfate concentrations of 60 to 105 μM extends the known range of sediment habitats in which sulfate reduction can be a dominant terminal process.

Anaerobic metabolism of organic matter in freshwater sediments has been studied primarily in eutrophic lake sediments where methanogenesis is the dominant terminal process. In such sediments, sulfate-reducing bacteria (SRB) can only outcompete methanogenic bacteria (MB) for hydrogen and acetate when sulfate is increased to millimolar concentrations (8, 18). At in situ sulfate concentrations, SRB utilize less than 10% of the hydrogen production and less than 15% of the acetate produced in eutrophic lake sediments (8, 9, 14). Total sulfate reduction rates, which may include the metabolism of substrates other than hydrogen and acetate by SRB, are less than 15% of methane production rates in eutrophic sediments (8, 20). Several studies have also suggested that SRB function as net producers of hydrogen and acetate in freshwater sediments (6, 18).

Eutrophic lake sediments represent only one extreme in the continuum of possible sediment environments in lakes. The sulfate gradient into eutrophic lake sediments is very steep (11, 13, 18), which confines the most active zone of

sulfate reduction to the most superficial sediments (5). Present methods are not adequate for sampling this zone or for maintaining in situ sulfate concentrations during experimental incubations. Since the inability to adequately sample the most active sulfate reducing zone in eutrophic sediments may have resulted in an underestimate of the ability of SRB to compete with MB in freshwater, the interactions between the two populations were investigated in the sediments of an oligotrophic lake in which the sulfate gradient in the sediments was less steep.

MATERIALS AND METHODS

Sediment sampling and incubation. Lawrence Lake is a small (maximum depth 12.5 m), oligotrophic, hard water lake located in southwestern Michigan. The physical, chemical, and biological characteristics of the lake have been described in detail (reference 17 and references therein). Sediments were sampled late in summer stratification from within the 12-m depth contour. Oxygen is depleted from the water overlying these sediments during this time (17). Sulfate concentrations in the interstitial water were determined from dialysis samplers (3) with ports spaced at 1-cm intervals. For measurements of methane production at various depths, sediments were collected with a gravity corer as previously described (9). Subcores were taken at 2-cm intervals with cut off 3-ml plastic syringes, which were then sealed with rubber stoppers and incubated underwater at the in situ sediment temperature during transport. The sediments were

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extruded into 10-ml Vacutainers (Becton, Dickinson & Co.) and treated as outlined below. In all subsequent experiments, sediments were sampled with an Eckman dredge. Care was taken to collect only the flocculent surface sediments and associated water. The sediments were transported to the laboratory on ice to slow sulfate depletion, and experiments were initiated within 1 h of sediment collection.

Sediments were transferred under a nitrogen atmosphere to anaerobic pressure tubes (Bellco Glass) or to 10-ml Vacutainers. Incubations were at 10°C in the dark. Methane production was measured as the rate of accumulation of methane over time. The tubes were vigorously shaken to equilibrate dissolved methane with the headspace before the gas samples were taken. Sulfate reduction rates were estimated from the rate of sulfate depletion in the sediment over a 3.5-h incubation period. Replicate samples were sacrificed over time and centrifuged to collect the interstitial water, which was analyzed for sulfate. Solutions of sodium molybdate (final concentration, 1 to 2 mM) and sodium sulfate were flushed with oxygen-free nitrogen and added to the sediments with a syringe and needle. The added volume was <1% of the total sediment volume.

For hydrogen uptake experiments, 100 ml of sediment was placed in a 120-ml serum bottle (Wheaton Scientific). Additions of chloroform (0.003% [vol/vol]), molybdate, or both were made before transferring 5-ml portions into pressure tubes. Preliminary experiments had demonstrated that chloroform and molybdate completely inhibited methane production and sulfate reduction, respectively. The sediments were preincubated for 1 to 3 h with slow rolling on a tube roller to create a thin film of sediment. Hydrogen was then added to the headspace to give an initial partial pressure of approximately 45 Pa, which was less than one-third of the half-saturation constants determined previously for hydrogen uptake by MB and SRB (9). Thus, short-term hydrogen uptake measured over a 30-min period was assumed to follow first-order kinetics, where the velocity of uptake equals the first-order rate constant, k , multiplied by the substrate concentration. Since it was not possible to start all hydrogen uptake measurements at exactly the same hydrogen partial pressure and since k is a direct function of the hydrogen-consuming populations, k was used to compare the effect of inhibitors on hydrogen uptake. The rate constant was calculated from $k = (H_0 - H_t) \times t^{-1} \times [(H_0 + H_t) \div 2]^{-1}$, where H_0 and H_t are the hydrogen partial pressures at the initial time point and at time t .

Solutions of radioactive compounds were pre-flushed with oxygen-free nitrogen. The volume of the added solution was 5% or less of the sediment volume, and the concentration of the added compounds was less than 2% of the in situ pool size. [2- 14 C]acetate (54 mCi mmol $^{-1}$) and [14 C]sodium bicarbonate (0.1 mCi mmol $^{-1}$) were purchased from New England Nuclear Corp.

The kinetics of acetate uptake were determined by the method of Wright and Hobbie (19). Various final concentrations of acetate were added to sediments along with [2- 14 C]acetate (2.6×10^5 dpm per 5 ml of sediment). The data were analyzed to the equation of Hobbie and Crawford (4):

$$t/f = (K + S_n)/V + A/V$$

where t is the incubation time, f is the fraction of the added activity converted to product, S_n is the in situ substrate concentration, A is the amount of substrate added, V is the maximum uptake velocity, and K is the substrate concentration that gives a half-maximal velocity of uptake. A plot of t/f versus A yields a line with an x-intercept of $-(K + S_n)$, if uptake follows Michaelis-Menten kinetics.

Analytical techniques. Methane concentrations were measured on a Varian 600 D gas chromatograph equipped with a flame ionization detector. Gases were separated with helium as the carrier gas on a 1-m column of Porapak N (Waters Associates) at 50°C. Hydrogen was analyzed on a Varian 3700 gas chromatograph equipped with a thermal conductivity detector. The gases were separated at 35°C on a 3-m column of Porapak N with nitrogen as the carrier gas at a flow rate of 15 ml/min. $^{14}\text{CH}_4$ and $^{14}\text{CO}_2$ were separated at 45°C on the same column, but with helium as the carrier gas. The effluent from the detector was passed through a gas proportional counter for quantification of the radioactivity. Total $^{14}\text{CO}_2$ in the tubes was corrected for dissolved ^{14}C inorganic carbon with an empirical factor that was determined from the distribution of [^{14}C]bicarbonate added to the sediment.

Sulfate was analyzed by high-pressure liquid chromatography. Ions were separated on a Vydac column (5 by 0.46 cm; Anspec) with a solvent of 1 mM phthalic acid (pH 5.5) at a flow rate of 2 ml/min at room temperature. Sulfate was detected with a conductivity detector (Anspec). Nitrate and nitrite in interstitial water collected by centrifugation were separated on a Partisil PXS 10/25 SAX column (Whatman), with 50 mM phosphate buffer (pH 3.0) as the solvent at a flow rate of 2.2 ml/min. The ions were detected by UV absorbance at 210 nm in a flow cell detector (Hitachi).

RESULTS

Methane production rates were highly variable among subcores taken at the same depth and time (Fig. 1). A high day-to-day variability was also noted, especially at the lower depths examined. Sulfate concentrations decreased rapidly with depth, indicating active sulfate reduction in the surface sediments (Fig. 1). To obtain sediments with the highest sulfate concentrations and to reduce variability within a sediment sample for a given date, all subsequent studies were performed with well-mixed surface sediments collected with an Eckman dredge.

The addition of molybdate to the sediment completely inhibited sulfate depletion (data not shown) and stimulated methane production (Table 1). The extent of molybdate stimulation of methane production was variable, reflecting the heterogeneity of the sediments and the difficulties in consistently sampling the same sediment interval from day to day. When samples of the sediment collected on 5 October were incubated for 1 h with [2- 14 C]acetate, molybdate inhibited the rate of $^{14}\text{CO}_2$ production over 90%, with a corresponding 74% increase in the production of

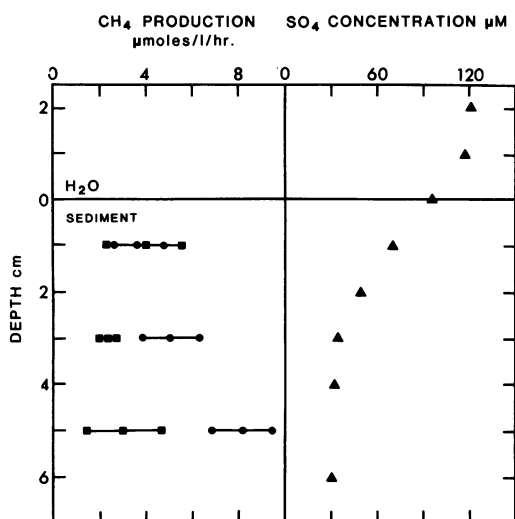


FIG. 1. Methane production (mean and range of standard error on 12 August (●) and 13 August (■)) and sulfate concentrations (▲) with depth on 24 August at the 12.5-m depth site of Lawrence Lake.

$^{14}\text{CH}_4$. The sediments collected on 9 October (Table 1) were incubated with ^{14}C sodium bicarbonate. The amount of $^{14}\text{CH}_4$ produced after the 25-h incubation period was 6.7-fold higher in molybdate-treated sediments than in untreated sediments, which indicated that molybdate also stimulated methane production from hydrogen and carbon dioxide. Molybdate had no significant effect on methane production in sediments that were preincubated to deplete the sulfate pool (Table 1).

The increase in the number of moles of methane produced in molybdate-treated sediments was 96% of the number of moles of sulfate reduced in untreated sediments (Table 2). Since

the electron equivalents required for the production of methane and the reduction of sulfate are equal (16), this indicates that carbon and electron flow that was blocked from going through sulfate reduction proceeded through methanogenesis.

From the information that the increase in methane production in molybdate-treated sediments corresponded to the rate of sulfate reduction in untreated sediments, it could be calculated from the data in Table 1 that sulfate reduction comprised from 30 to 81% (mean, 50%) of the total carbon and electron flow proceeding through methane production and sulfate reduction. Direct simultaneous measurements of initial rates of sulfate reduction and methane production in early October yielded rates of 4.6 μmol of sulfate reduced and 1.8 μmol of methane produced per liter of sediment per hour. Thus, SRB metabolized greater than twice the number of electron equivalents metabolized by MB in these samples. Of the total $[2-^{14}\text{C}]$ acetate converted to $^{14}\text{CO}_2$ and $^{14}\text{CH}_4$, the percentage metabolized to $^{14}\text{CO}_2$ was 86% on this date (5 October) and ranged from 40 to 97% (mean, 74%) throughout the study period.

The uptake of added hydrogen was inhibited by chloroform or molybdate alone, and the effect of the inhibitors was additive (Table 3). However, hydrogen uptake continued even when methane production and sulfate reduction were completely inhibited, which indicated that other processes could utilize hydrogen at increased hydrogen partial pressure.

Kinetic analysis of $[2-^{14}\text{C}]$ acetate metabolism indicated that the $K + S_n$ value for $^{14}\text{CO}_2$ production, 5 μM , was significantly lower than the $K + S_n$ value of 33 μM for $^{14}\text{CH}_4$ production (Fig. 2). The sulfate concentration in these sediments was 60 μM . The greater than 90% inhibition of $^{14}\text{CO}_2$ production from $[2-^{14}\text{C}]$ acetate

TABLE 1. Effect of molybdate and sulfate additions on methane production in Lawrence Lake surface sediments

Sediment sample	Incubation period (h)	Initial sulfate concentration (μM)	No. of replicates per treatment	Methane production rate in untreated sediment ($\mu\text{mol/liter per h}$) ^a	Methane production rate ratio with: ^b	
					MoO_4^{2-}	SO_4^{2-} ^c
7 September	25	ND ^d	4	2.48 ± 0.61	1.42	ND
23 September	21.5	85	5	5.85 ± 1.96	1.45	0.50 (125 μM)
26 September	8.25	72	4	3.00 ± 1.11	1.54	0.75 (2.5 mM)
5 October	20	105	5	0.97 ± 0.09	3.91	ND
9 October	25	ND	3	1.44 ± 0.03	5.35	ND
Sulfate depleted ^e	22.75	<2	5	2.29 ± 0.35	1.06	ND
Sulfate depleted	4.75	<2	5	3.16 ± 0.77	1.02	ND

^a Mean \pm standard error.

^b (Methane production in treated sediment) \times (methane production in untreated sediments)⁻¹.

^c Concentration of the added sulfate is shown in parentheses.

^d ND, Not determined.

^e Sediment incubated in the dark to deplete the sulfate pool.

TABLE 2. Methane production and sulfate reduction in control and molybdate-treated Lawrence Lake sediments over a 20-h incubation period^a

Sediment	Methane produced (μmol/liter)	Sulfate reduced (μmol/liter)
Control	19.4 (1.8)	58.9 (2.9)
Molybdate treated	75.7 (8.2)	0
Difference ^b	-56.3	58.9

^a Mean with standard error in parentheses; $n = 5$ for methane production, $n = 4$ for sulfate reduction.

^b (Control sediments) - (molybdate-treated sediments).

reported above indicated that the kinetics of acetate metabolism to $^{14}\text{CO}_2$ primarily represented the kinetics of the SRB. The conclusion that sulfate was the electron acceptor for acetate oxidation was supported by the observation that nitrate and nitrite were not available as electron acceptors. The nitrate and nitrite pools in this sediment were 0.6 and 2.0 μM, respectively, and these pools did not decrease when sediments were incubated under anaerobic conditions. To further ensure that the kinetics for $^{14}\text{CO}_2$ production were due to sulfate reduction, sediments were preincubated in the dark for approximately 2 weeks to deplete electron acceptors for acetate oxidation. The sediments were then amended with 500 μM calcium sulfate 3 days before kinetic analysis. The sulfate concentration on the day of the analysis was 350 μM. The $K + S_n$ values for $^{14}\text{CH}_4$ and $^{14}\text{CO}_2$ production were 65 and 3 μM, respectively.

DISCUSSION

The results demonstrated that SRB could effectively compete with MB for hydrogen and acetate at the in situ sulfate concentrations found in the surface sediments of an oligotrophic lake. This was indicated most directly by the stimulation of methane production after the inhibition of sulfate reduction with molybdate. The conclusion that molybdate stimulated methane production by alleviating SRB competition for hydrogen and acetate was demonstrated by: the correspondence between the rate of sulfate reduction in control sediments and the increased rate of methane production in molybdate-treated sediments; the molybdate stimulation of $^{14}\text{CH}_4$ production from $[2-^{14}\text{C}]$ acetate and $^{14}\text{CO}_2$; and the lack of an effect of molybdate on methane production in sulfate-depleted sediments.

The stimulation of methane production associated with the inhibition of sulfate reduction in Lawrence Lake is similar to that observed in marine sediments, in which SRB consume a significant proportion of the hydrogen and ace-

TABLE 3. Effect of inhibitors on the uptake of added hydrogen in Lawrence Lake sediments

Inhibitor added	k (h ⁻¹) ^a	% Inhibition
None	0.74 ± 0.14	
Chloroform	0.55 ± 0.02	26
Molybdate	0.55 ± 0.05	26
Chloroform and molybdate	0.39 ± 0.06	47

^a Mean ± standard error of the first-order rate constant for uptake, k , for triplicate determinations. The rate of hydrogen uptake in sediment with no inhibitors added and approximately 45 Pa of hydrogen partial pressure was 60 μmol/liter of sediment per hour.

tate production (1, 12, 15), but contrasts with the lack of stimulation in eutrophic lake sediments, in which SRB are of relatively minor importance in acetate and hydrogen metabolism (14). Inhibition of sulfate reduction with B-fluorolactate previously has been shown to slightly stimulate methane production in Lake Vechten sediments during long-term incubations (2). Although the increased methane production in that study was attributed to the lower concentrations of hydrogen sulfide in the sediments in which sulfate reduction was inhibited, the results are also consistent with a competition in situ between SRB and MB for acetate and hydrogen. A decrease in sulfide toxicity to MB was not a factor in the short-term experiments conducted with Lawrence Lake sediments, in which the in situ concentration of dissolved sulfide is less than 3 μM, and high concentrations of dissolved ferrous iron precipitate most of the sulfide produced.

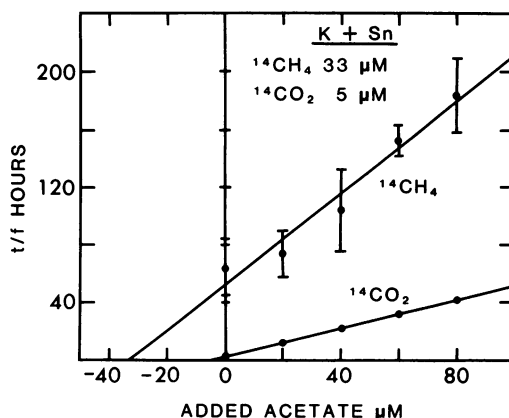


FIG. 2. Metabolism of $[2-^{14}\text{C}]$ acetate in Lawrence Lake surface sediments at different concentrations of added acetate. Error bars represent standard error of the mean. Standard error for $^{14}\text{CO}_2$ is contained within the area of the symbols.

The lower SRB half-saturation constant for acetate uptake permits SRB to successfully compete with MB for acetate in Lawrence Lake sediments. A comparison of the data of Lawrence and McCarty (7) and Middleton and Lawrence (10) on the acetate growth kinetics of MB and SRB, respectively, supports the conclusion that SRB have a higher affinity for acetate than MB and also suggests that SRB have a higher maximum rate of acetate utilization per unit biomass and a higher growth yield. These factors should further increase the advantage of SRB in competing with MB for acetate.

Previous studies have demonstrated that freshwater SRB have a lower K for hydrogen than MB when sulfate is not limiting (8). However, the previously employed approach of using molybdate and chloroform as selective inhibitors for sulfate reduction and methane production could not be used to determine the K of MB and SRB for hydrogen uptake in Lawrence Lake sediments since there was a potential hydrogen-consuming population(s) other than SRB and MB at elevated hydrogen partial pressures. The nature of this hydrogen-consuming reaction(s) and its significance at the in situ hydrogen partial pressure of approximately 1 Pa is unknown. The stimulation of methane production from hydrogen after the addition of molybdate did, however, indicate that SRB could effectively compete with MB for hydrogen at the low sulfate concentrations of Lawrence Lake sediments.

The actual importance of sulfate reduction as a terminal process in Lawrence Lake sediments was underestimated by the methods employed in this study. The sulfate concentration in the sediment limited the uptake of acetate and hydrogen by SRB since the addition of sulfate inhibited methane production (Table 1) and since no methane should be produced from acetate and hydrogen if sulfate is not limiting to SRB (8, 18). The sulfate concentrations decreased during the incubation of the sediment, thus further diminishing the ability of the already sulfate-limited SRB population to compete with MB. The rapid depletion of sulfate in sediment samples isolated from the sulfate supply of the overlying water and the steep gradient of sulfate concentration in the sediment require that rate measurements on cores of undisturbed sediment and overlying water be made before the actual contributions of sulfate reduction and methanogenesis to total sediment metabolism can be determined.

The results reported here indicate that findings from eutrophic lake sediments cannot be extrapolated to the sediments of less productive lakes. SRB have the potential to be the dominant acetate and hydrogen consumers in the surface sediments of freshwater lakes. These findings

stress the importance for future studies on the metabolism of sedimented organic matter in oligotrophic and mesotrophic lakes and on the factors that control the relative importance of sulfate reduction and methane production in the sediments of lakes in various trophic states.

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